## REMARKS UNDER 37 CFR § 1.111

### Formal Matters

This paper is responsive to the Office Action dated October 3, 2002 (Paper No. 5), which is the first action on the merits of the application.

Claims 31-62 were previously pending in the application, and under examination.

As a result of entering this Amendment, certain claims are amended, claim 51 is canceled without prejudice, and claims 63-81 are added. The new claims cover pharmaceutical compositions comprising a cell expressing a membrane cytokine, and therefore fall within the same category as the claims previously pending. Accordingly, claims 31-50, and 52-81 are now under examination.

No new matter has been added to the disclosure as a result of entering these amendments.

Reconsideration and allowance of the application is respectfully requested.

### Interview:

The applicants wish to thank Examiner Christopher Yaen for a constructive and helpful telephone interview with Carol Francis and Michael Schiff on February 27, 2003. Recommendations made by Dr. Yaen are incorporated into this response.

### Amendments to the claims:

No new matter is added to the disclosure as a result of entering this paper into the application file. Support for the amendments and the new claims can be found throughout the application as originally filed, including the following:

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| Claim 31: | Claims 31 and 44 as previously presented; page 31 lines 17-19 |
| Claims 34, 36-39, 41, 42 and 59-61: | Claim 51 as previously presented |
| Claim 44, 45, and 49: | Page 20, lines 5-10 |
| Claim 63: | Claim 44 as previously presented |
| Claim 64: | Page 20, lines 5-10 |
| Claims 65-67: | Claim 32 as previously presented |
| Claims 68-81 | Claims 31--34, 38-42, 45, 55, and 56 as previously presented, and page 20, lines 5-10 |

Reference to the cytokine-producing cell has been changed from the wording originally presented (a genetically altered cell) to a cell that expresses a cytokine from a recombinant polynucleotide. This has been done to facilitate the wording of the rest of the amended claims. The skilled reader will appreciate that the polynucleotide referred to can be a vector that is resident in the cell (such as an adenovirus: page 30, lines 4-18), or a recombinant nucleic acid introduced into the genome (such as by a retroviral vector: page 30, line 19 to page 31, line 16). The cell can be the same cell initially transfected with the vector, thereby containing the recombinant polynucleotide, or a progeny thereof that has inherited the recombinant polynucleotide.

**Rejections under 35 USC § 112 ¶ 2:**

Various claims were rejected under §112, ¶2. Each of these rejection is addressed below.

Claims 31, 40-41, 45, 46 55, 62 and dependent claims thereon were rejected for recitation of "associated with the cell outer membrane" or "membrane-associated." Applicants respectfully disagree. The meaning of "a cytokine stably associated in the outer membrane" is clear from the specification, and further that this terminology would be readily understood by one of ordinary skill in the art upon reading the specification. For example, specification page 21, lines 16-19 indicates that a transmembrane protein is an example of a protein that "remains stably associated in the membrane of the cell". Furthermore, at page 26, line 24 to page 27, line 20, describes various exemplary associations a cytokine may have with the cell from which it is produced. The cytokine is generally attached to the cell membrane so as to keep it in the vicinity of bystander tumor antigen (page 26, lines 25-27).

In one example, the cytokine is a fusion protein comprising a heterologous transmembrane region which provides for its stable association with the cell membrane (page 27, lines 12-20). In another example, the cytokine is a naturally occurring membrane form of a cytokine that is synthesized in one or more different isoforms (see, *e.g.*, Example 5, page 61, line 1 to page 63, line 25). The ordinarily skilled artisan will readily recognize that all the variants of a cytokine stably associated with the membrane that are outlined in the Office Action are included in the invention. Applicants note that this same rejection was made in the parent

application (U.S. application serial no. 08/901,225, filed July 24, 1997, now U.S. Pat. No. 6,277,368), and was overcome by these same arguments presented above.

Claims 31, 38, 41, and claims dependent thereon stand rejected as indefinite for referring to a composition "effective in treating" the disease or eliciting an immunological response. Applicants respectfully disagree. This phrase is common terminology in issued U.S. patents, and will be clear to the clinician or immunologist managing the subject's care. Treatment is defined in the specification on page 21, and desirable outcomes of treatment are exemplified on pages 34-38. Accordingly, the term complies with the requirements of § 112 ¶ 2. The actual dose and formulation of the composition will depend on the nature of the tumor and condition of the patient, and will be optimized empirically. This is an objective of Phase II and Phase III human clinical trials.

Claim 49 stands rejected as unclear for referring to a "tumor associated antigen". Applicants respectfully disagree. The term is extensively defined and described on page 21, lines 5-15. As exemplified elsewhere in the specification, the antigen may be present in the composition expressed on a cell, or obtained from a cell that expressed it.

Claim 34 stands rejected as indefinite for alternately referring to "tumor" and "cancer". Of course, not all tumors are cancerous. This claim has now been amended to use the term "tumor" throughout.

The amendments made under this section do not narrow the claim scope. Accordingly, these claims protect equivalents by an extent to which they are otherwise entitled.

Withdrawal of these rejections is respectfully requested.

**Rejection under 35 USC § 112 ¶ 1:**

All claims under examination stand rejected under this section as being enabling for a composition comprising a cell expressing a membrane M-CSF, but not for any composition comprising a cell expressing any cytokine associated with the cell outer membrane or, therefore, for treating neoplastic disease.

Applicants respectfully disagree. The Office Action seeks to limit coverage to the working examples. Of course, this is not the legal standard. The specification need not provide actual data for all possible species that fall within the genus. It is sufficient for the specification to provide procedures for carrying out the invention, which in combination with reagents and procedures otherwise known in the art, allow someone skilled in the art to practice the claimed invention without undue experimentation.

Page 26, line 24 to page 27, line 20 provides an extensive discussion of membrane-associated cytokines, and how cytokines that are normally secreted can be altered so as to be expressed in a membrane-associated form. For example, it tells the reader that cells can be genetically altered with a vector containing a cytokine encoding region and a transmembrane region in the same open reading frame. The transmembrane region may be modeled on other known transmembrane proteins, or an artificially designed polypeptide segment with a high degree of lipophilicity.

Accompanying this response is an article by W. Soo Hoo et al., entitled *Tumor cell surface expression of GM-CSF elicits antitumor immunity and protects from tumor challenge in the P815 mouse mastocytoma tumor model.* J. Immunol. 162:7343-7349, 1999. The authors followed the procedure provided on page 27 of the specification using the pHOOK-1 plasmid vector, commercially available from Invitrogen. The vector as sold apparently already contains the PDGF receptor transmembrane domains, and restriction nuclease sites for inserting a heterologous extracellular domain. The authors of this paper ligated the GM-CSF encoding region into the vector using standard techniques. There is no indication that the procedure was particularly difficult, or that the authors needed to test other vectors or other transmembrane regions before selecting the conditions used. In fact, the entire procedure of amplifying out the GM-CSF encoding region from an expression library and cloning it into the pHOOK-1 vector is viewed as so straight-forward by the authors that it occupies only two paragraphs in small type in the methods section (page 7344, col. 1).

Soo Hoo et al. were apparently able to duplicate the procedure provided in the specification without undue experimentation. Accordingly, the specification is enabling for cells expressing cytokines in a membrane-associated form, even when the cytokine is normally secreted. Withdrawal of this rejection is respectfully requested.

**Rejections under 35 USC § 102:**

**Kimura et al.**

Certain claims in the application stand rejected under § 102(a) as being anticipated by Kimura et al., Exp. Hematol. 24:360, 1996. The Office Action indicates that Kimura et al. teach a method of stimulating an immune response using a cellular composition comprising M-CSF (purportedly in the membrane-associated form), along with other features recited in the claims.

Applicants respectfully disagree. In the work described in the article, live L1210 tumor cells were administered into syngeneic mice, where they grew to a lethal size. When the cells were expressing M-CSF, the animals showed improved survival. There is no affirmative indication in the article that the M-CSF was present in a membrane associated form — in fact, it is referred to on page 361, col. 1 in soluble form (20 μg/kg), and levels of M-CSF of mice administered with M-CSF expressing cells was subsequently measured in serum (page 361, col. 2). It is unclear whether the M-CSF expressing cells is being administered in combination with tumor antigen in a manner that elicits an immune response. Only survival data are given. It is also unclear how (if at all) this system should be adapted to human clinical therapy.

In contrast, Example 6 (page 61 ff.) of this application compares the immunogenic effect of cancer cells expressing a membrane-associated form of M-CSF, with cells expressing a secreted form of M-CSF. Animals receiving the membrane-associated form had considerably better survival times than those receiving the secreted form (page 62, lines 22-25). Moreover, rejection of cells amongst various clones correlated with the level of membrane expression of M-CSF (page 62, line 25 to page 63, line 2). Evidence of a tumor-specific immune response was obtained by showing that the immunized animals were protected against a rechallenge with parental T9 glioma cells, but not mammary adenocarcinoma cells (page 63, lines 3-10). Furthermore, protection could be transferred from one animal to another by way of a lymphocyte-containing cell population (page 63, lines 11-17).

Base claims 31 and 68 of this application now incorporate the limitation previously presented in Claim 51: The cell expressing the membrane cytokine is specified to be a human cell, so that the composition is suitable for treating human subjects. Claims 31, 49, and their dependents are further distinguished from the article by Kimura et al., because these claims require that the cell expressing the cytokine be inactivated to prevent proliferation. This feature is not taught or suggested in the article, since live cells were used. There is no suggestion in the

article that the cells can be inactivated in such a way that they are unable to proliferate, but still remain viable and continue to synthesize the recombinant cytokine. In contrast, this patent application describes how the cells can be irradiated with a titrated dose to prevent proliferation, but still synthesize cytokine (page 47, line 15 to page 48, line 24; page 51, lines 14-22; page 53, lines 21-25; page 59, lines 14-23).

Claims 36, 60, and 68 and its dependents are further distinguished from the article by Kimura et al., as discussed during the interview, because the claim requires that the cell expressing the cytokine be allogeneic to the treated subject. It is reasonable to surmise that the L1210 cells used in the article were syngeneic to the animals they were administered to, because they grew to form a tumor of lethal size without being rejected. Transfecting the cells with M-CSF apparently affected the viability or proliferation of the cells themselves in vivo. In contrast, the invention claimed in this patent application uses cytokine-expressing cells to recruit a host response against a tumor that may already be established in the host. In this manner, the immunizing cells are different from the cells against which the response is desired. The immunizing cells can be autologous or allogeneic to the subject being treated. In the embodiment of Claims 36, 60, and 68, the immunizing cells are necessarily allogeneic.

Applicants do not concede that this publication is valid prior art under § 102(a). Applicants also do not concede that the article describes the features recited in the dependent claims in this application. These points will not be addressed further, because the arguments presented already are sufficient to overcome the rejection.

**Jadus et al.**

Certain claims in the application stand rejected under 35 USC § 102(a) as being anticipated by Jadus et al., Blood 87:5232, 1996. The Office Action indicates that Jadus et al. teach a method of killing tumor cells comprising administering a composition expressing M-CSF.

Applicants respectfully disagree. First of all, the reference does not qualify as § 102(a) prior art. The scientists in the Jadus group and the inventors on this patent application collaborated at U.C. Irvine in the preparation of some of the reagents used. Graf and Hiserodt are acknowledged in the article on page 5233, col. 1, paragraph 1. Jadus is acknowledged in the patent application on page 62, line 5. Glioma cell line T9 transduced with the LXSN retrovirus

expression vector were used in both cases. Description of preparation of the cells on page 5233 of the article mirrors the description in the patent application on pages 61-62. The use of the cells in the article and in the patent application is directed to different purposes.

The article also differs from the claimed invention on a substantive basis. Jadus et al. report that macrophages killed hybridoma cells or T9 glioma cells expressing membrane M-CSF (Figure 5). The killing was inhibited by adding M-CSF to the medium (Table 2). The macrophage kill the cells directly (Figure 7), by binding M-CSF (macrophage colony stimulating factor) through receptors on the macrophage surface. This does not constitute administration of cytokine expressing cells to a subject. Instead, the cytokine expressing cells are used as target cells in a tissue culture experiment. Since the reaction consists of macrophages directly attacking target cells, it does not constitute an immunological response, which classically involves specific antibody or antigen-specific cells (T or B lymphocytes).

Claims 31, 49, and their dependents are further distinguished from the article by Jadus et al., because these claims require that the cell expressing the cytokine be inactivated to prevent proliferation. Claim 68 and its dependents are further distinguished from the article by Jadus et al., because it requires that the cell expressing the cytokine be allogeneic to the treated subject.

**Tuck et al.**

Certain claims stand rejected under 35 USC § 102(a) as being anticipated by Tuck et al. *Blood* 84:2182-8, 1994. The Office Action indicates that Tuck et al. teach a COS-1 cell expressing a M-CSF cytokine, which can be either membrane associated or soluble in its natural form, and further a method of producing a cell which expresses m-CSF.

Applicants respectfully disagree. Tuck et al. describe *in vitro* studies of M-CSF expression of cells in culture, with a view to characterizing the protein isotypes that are formed, and where they are located in the cell. The findings "underscore the importance of locally presented growth factors to hematopoietic stem cell differentiation and growth" (conclusion of the article, page 2187, col. 1). Applicants are unable to identify any suggestion in the paper that cells expressing membrane M-CSF would be useful as a pharmaceutical composition for treating cancer, or for any other therapeutic purpose. There is no suggestion to combine the M-CSF expressing cells with a pharmaceutical excipient, or to make any of the cell preparations while

taking the precautions needed to obtain a composition that is adequately sterile, sufficiently free of contaminants, and otherwise formulated for human administration.

In contrast, Example 5 of the specification (page 61 ff.) shows how membrane M-CSF expressing cells limit tumor growth, increase survival, and protect against rechallenge. Example 7 (page 64-71) shows how cytokine expressing cells can be evaluated and optimized in clinical trials of human cancer. Pages 32-34, 38-41, and 66-68 provide detailed information on the formulation, testing, and use of cytokine secreting cells in human administration.

Thus, unlike the information provided in this patent disclosure, Tuck et al. neither suggest nor provide any motivation for making a pharmaceutical composition that, upon administration to a subject, is effective in treating a neoplastic disease or eliciting an anti-tumor immunological response. Accordingly, this reference does not constitute prior art against the patentability of the claimed invention under either § 102 or § 103.

Withdrawal of all prior art rejections is respectfully requested.

**Conclusion**

Applicants respectfully request that all outstanding rejections be reconsidered and withdrawn.

Applicant submits that all of the claims are in condition for allowance, which action is requested. If the Examiner finds that a telephone conference would expedite the prosecution of this application, please telephone the undersigned at the number provided.

The Commissioner is hereby authorized to charge any underpayment of fees associated with this communication, including any necessary fees for extensions of time, or credit any overpayment to Deposit Account No. 50-0815, order number IRVN-001DIV.

Respectfully submitted,
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Enclosure:

W. Soo Hoo et al., *Tumor cell surface expression of GM-CSF elicits antitumor immunity and protects from tumor challenge in the P815 mouse mastocytoma tumor model.* J. Immunol. 162:7343-7349, 1999

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